Lecture 11
Fluorescence

by phytoplankton pigments and CDOM

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23 July 2021

Take Home Message

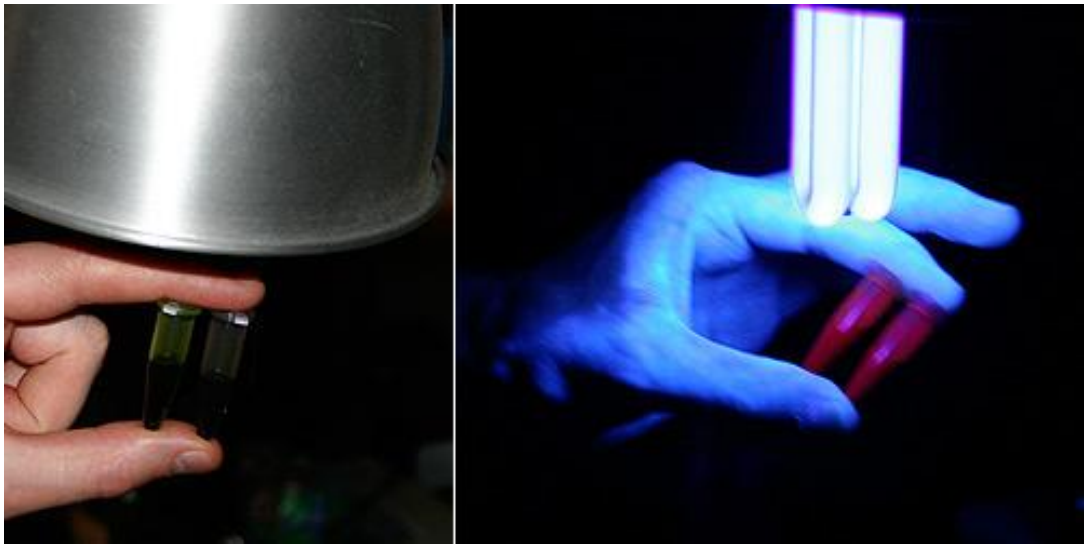
- Fluorescence is the property of a molecule to re-emit absorbed light energy at a longer wavelength (lower energy)
- Only certain molecules can fluoresce (e.g., chlorophyll a, some organic molecules), unique fingerprint
- Easy to measure, difficult to interpret (conservation of misery)
  - CDOM – complex composition
  - Chl – proxy for biomass, carbon, photosynthesis

Fluorescence

- What is it
- Who does it
- Physics of fluorescence
- Fluorescence proxies
  - *In vitro*
  - *In vivo*
  - *In situ*
- Calibration/Validation
- Given sources of variability, what can we learn?

In vitro chlorophyll fluorescence

- Extract appears green under ambient light
- When exposed to 440 nm light, appears red

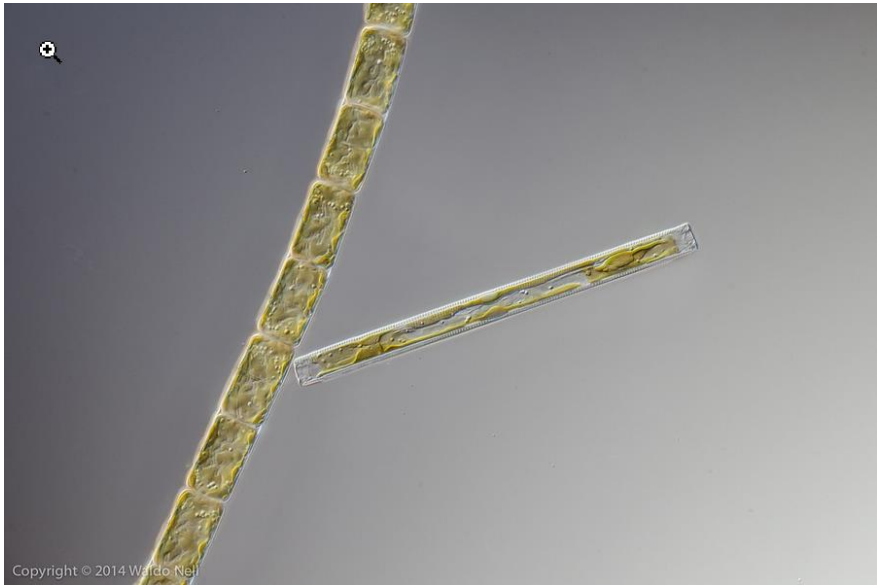


Credit Laura Cinti, http://c-lab.co.uk

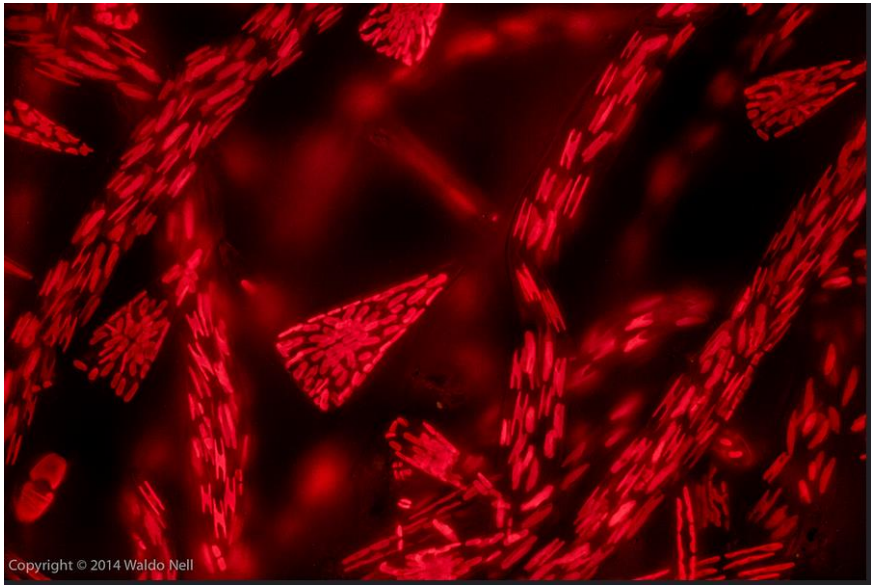
"Fluorescence of chlorophyll under UV light" by Marie Franzen http://commons.wikimedia.org/wiki/File:Fluorescence_of_chlorophyll_under_UV_light.jpg#

In vivo chlorophyll fluorescence

Light micrograph



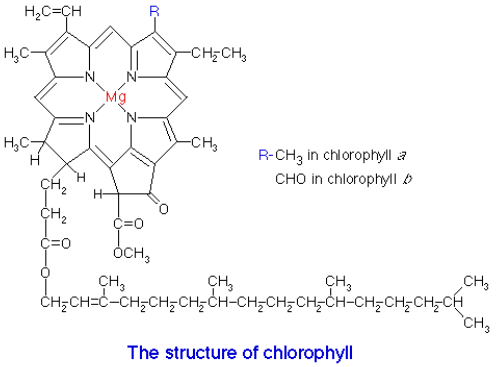
epifluorescent microscopy

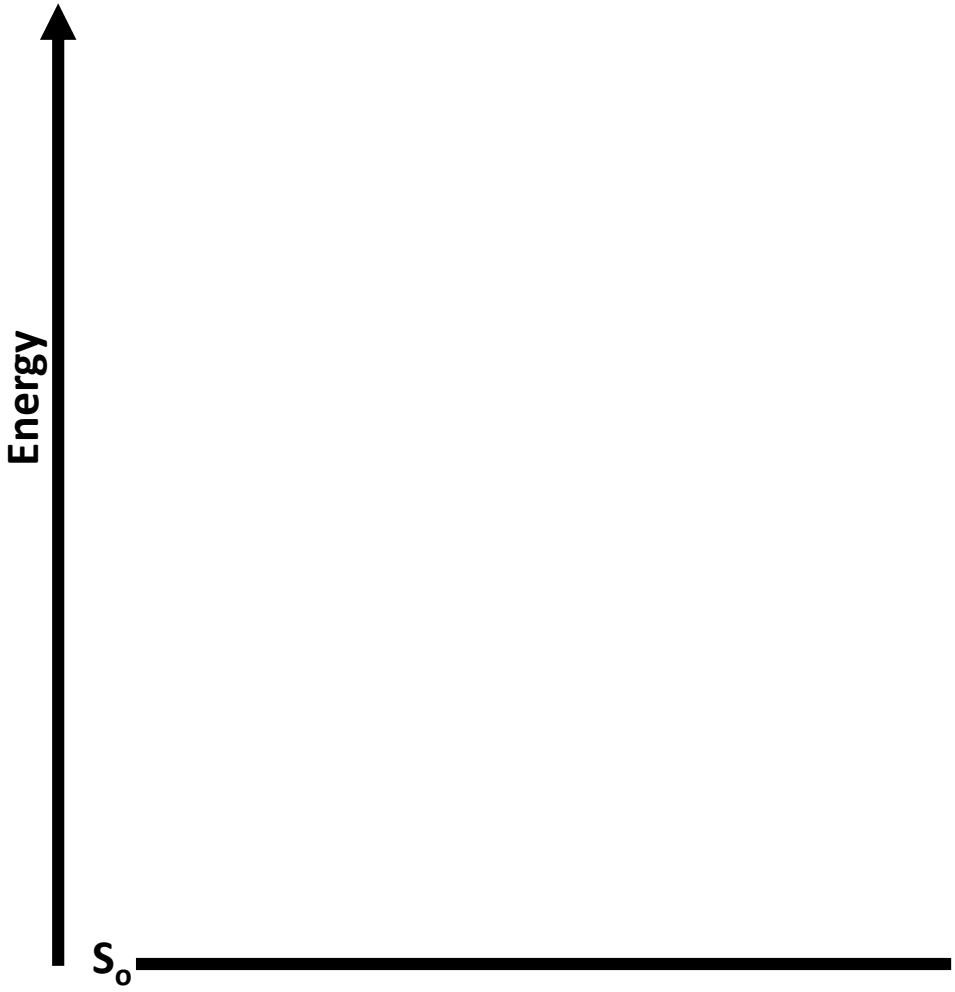


Fluorescence

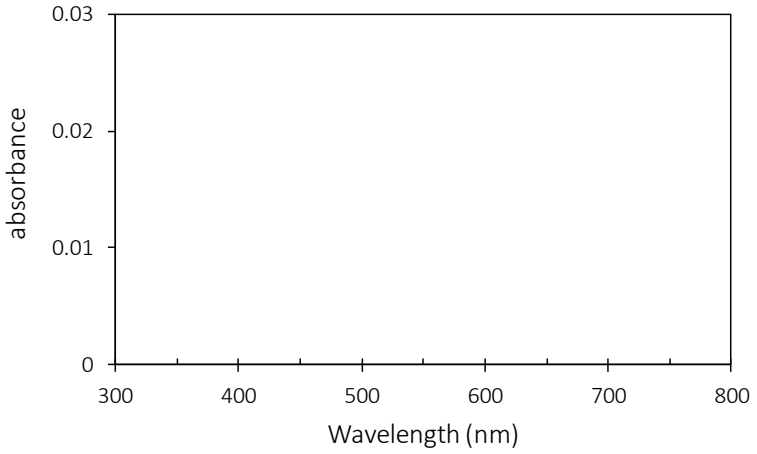
- What is it
- Who does it
- Physics of fluorescence
- Fluorescence proxies
  - *In vitro* – signal from chlorophyll in solvent extract
  - *In vivo* – signal from a living cell
  - *In situ* – bulk signal from the environment
- Validation
- Given sources of variability, what can we learn?

Review of electronic and vibrational states for Chlorophyll (Jablonski diagram)





- Sketch the electronic energy levels for the two absorption peaks for Chlorophyll a
- Use color-coded arrows to represent absorption from the ground state to each electronic state
- Draw the associated spectrum



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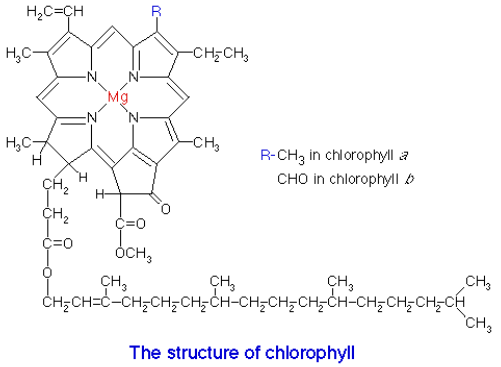

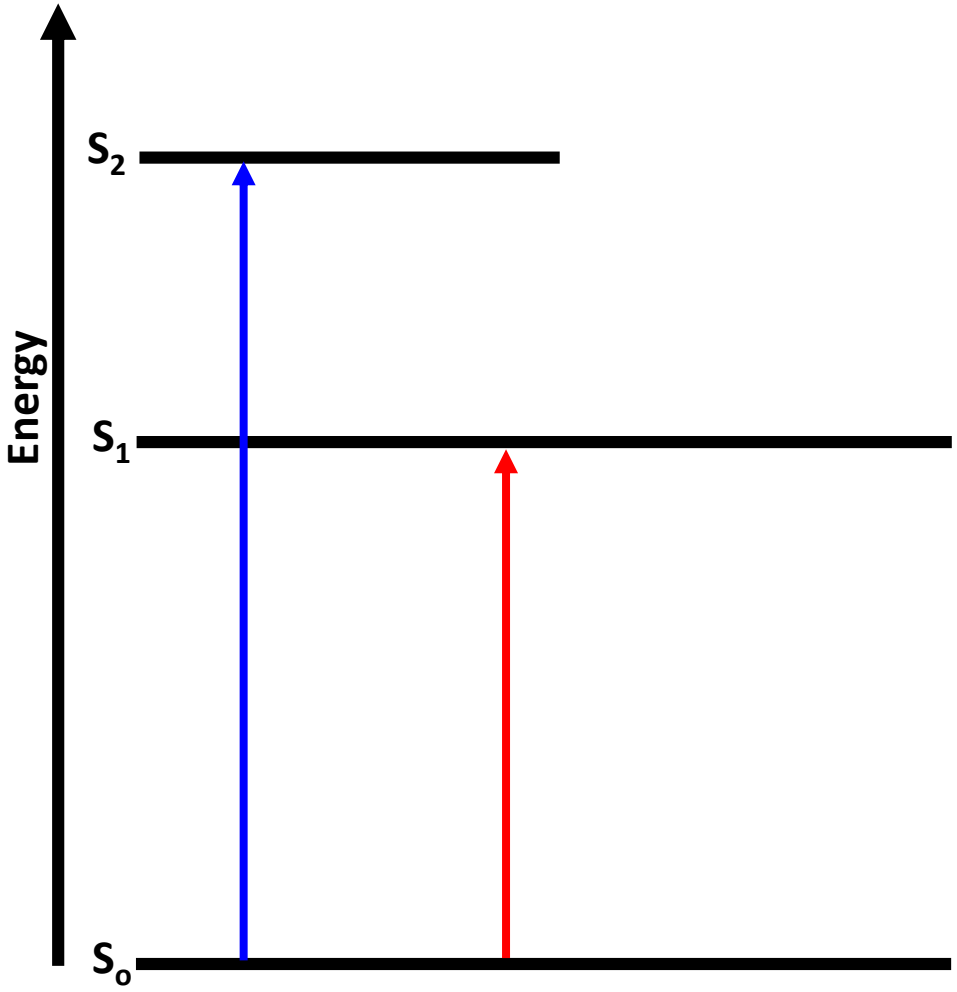

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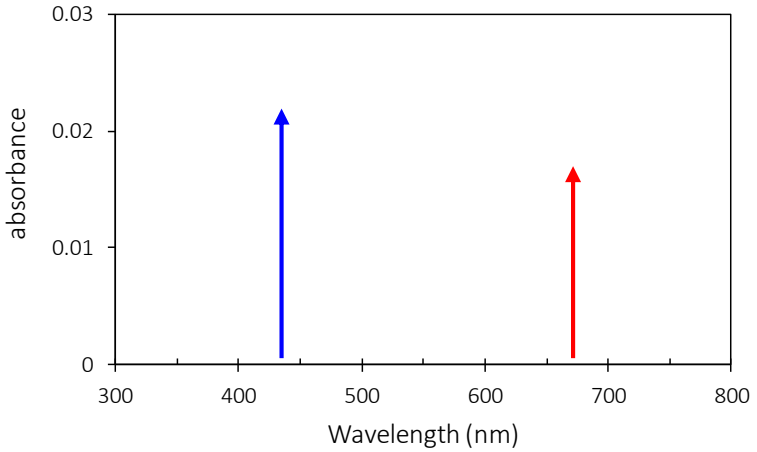

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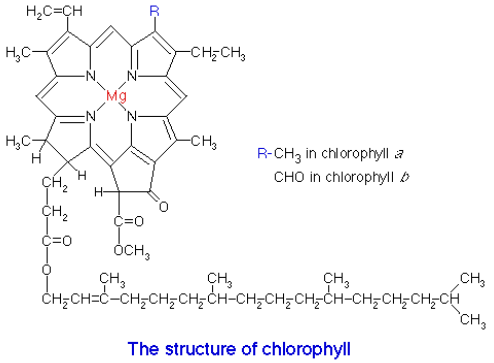
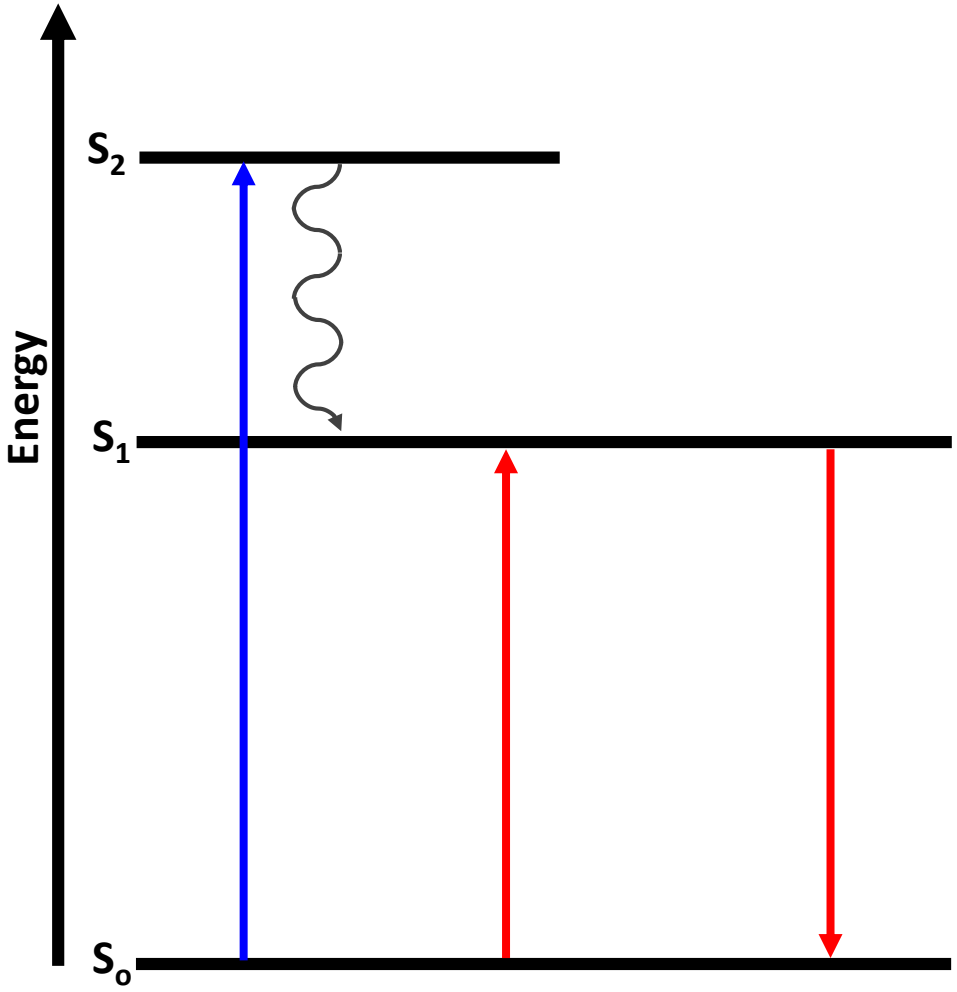
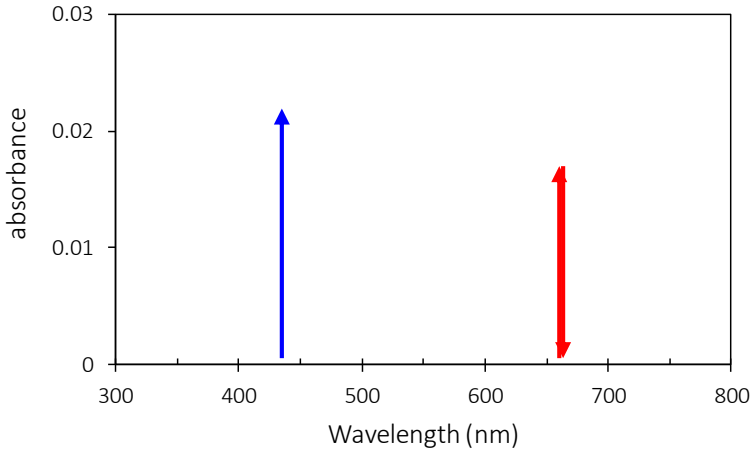


The structure of chlorophyll



- What happens to the absorbed energy?
- absorbed blue light is excess energy for photosynthesis, during energy transfer, the excess is lost as heat→ from S_2 to S_1
- What about the energy from S_1?
- Fluorescence emission, draw arrow to represent it on energy diagram and spectrum



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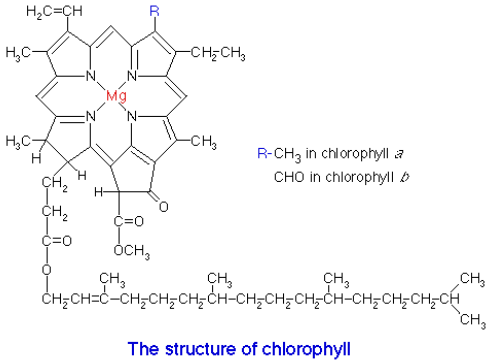

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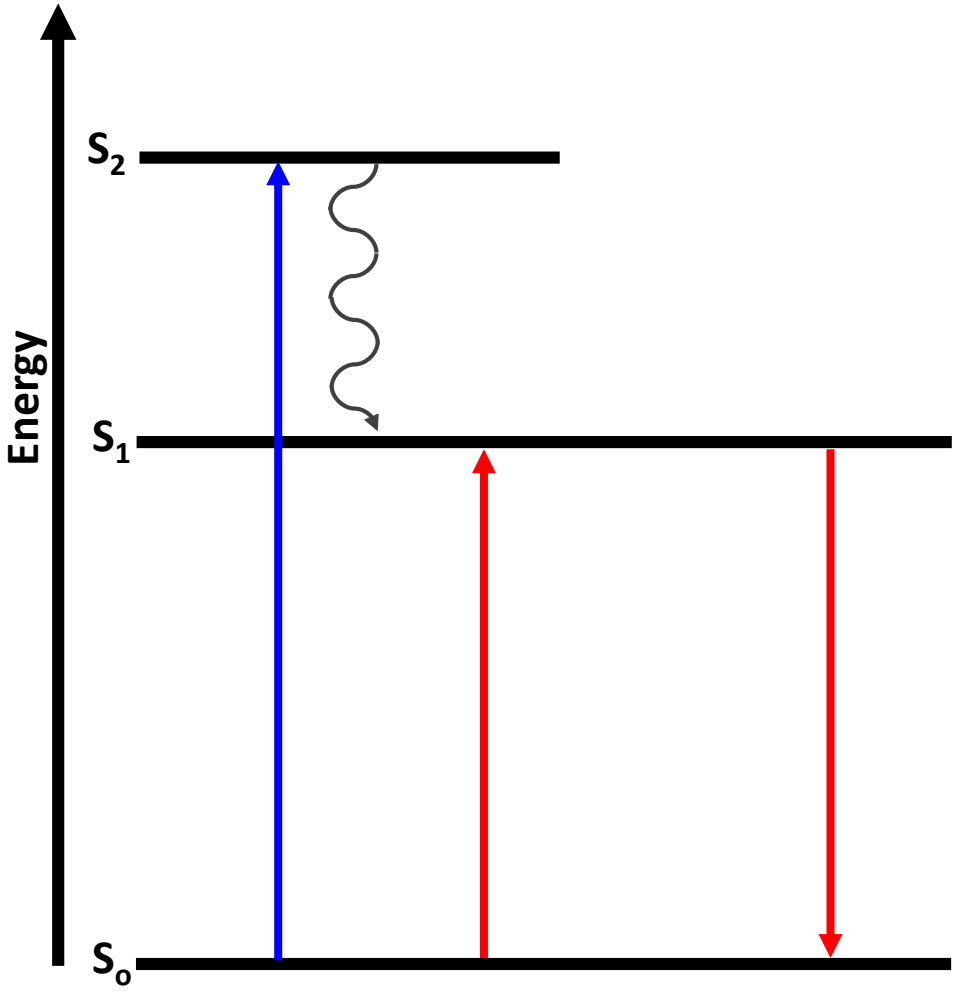
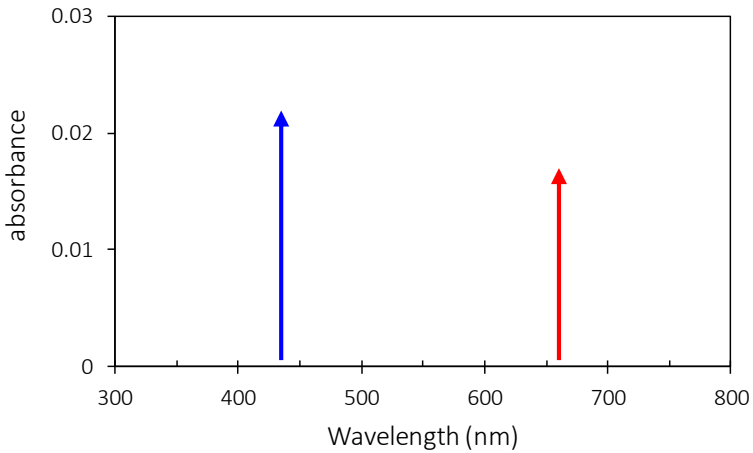


- Add 3 vibrational levels for each electronic state
- Add associated absorption to vibrational energy levels, spectrum
- Heat loss from vibrational levels to lowest level of electronic state
- Heat loss from S_2 to S_1



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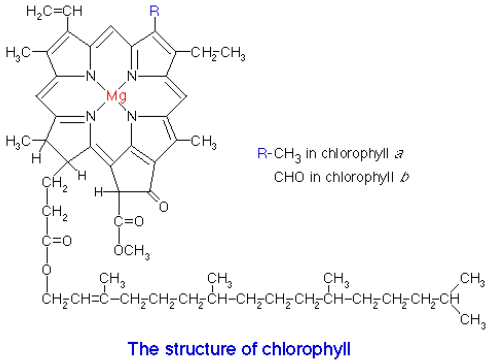

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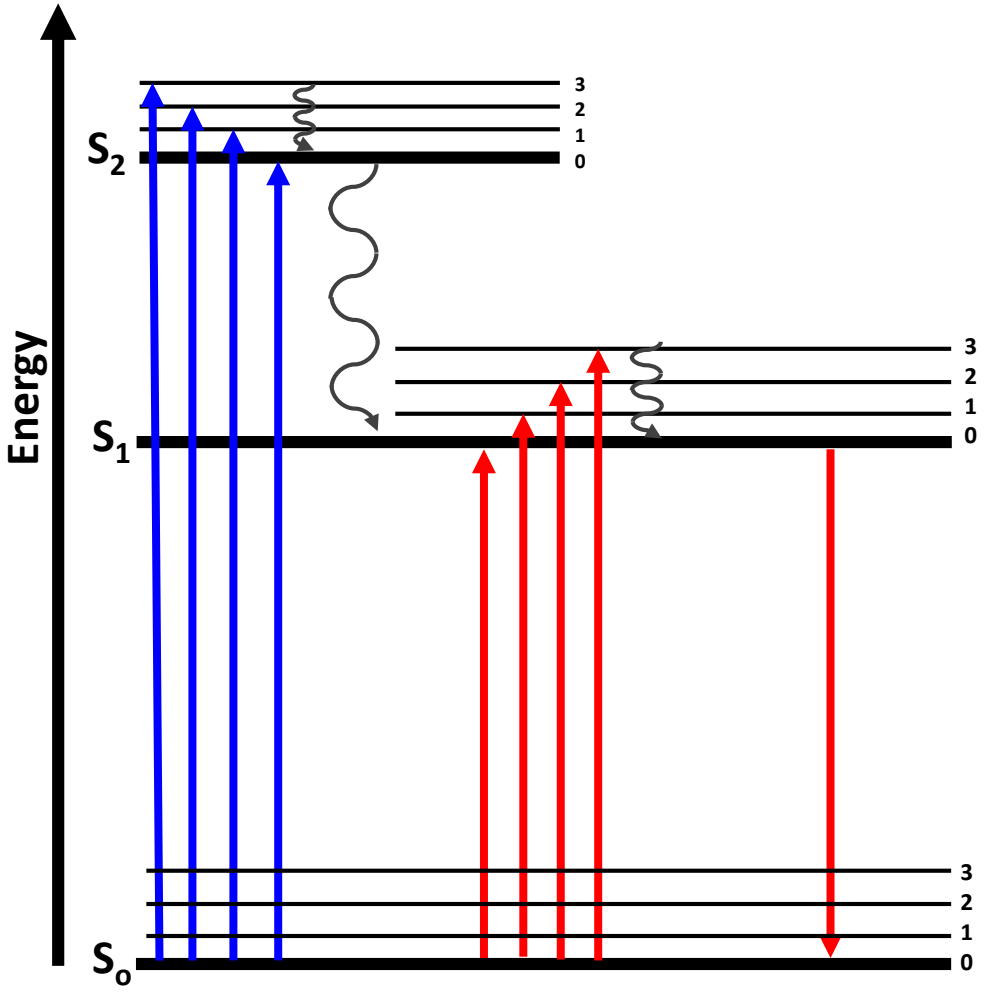

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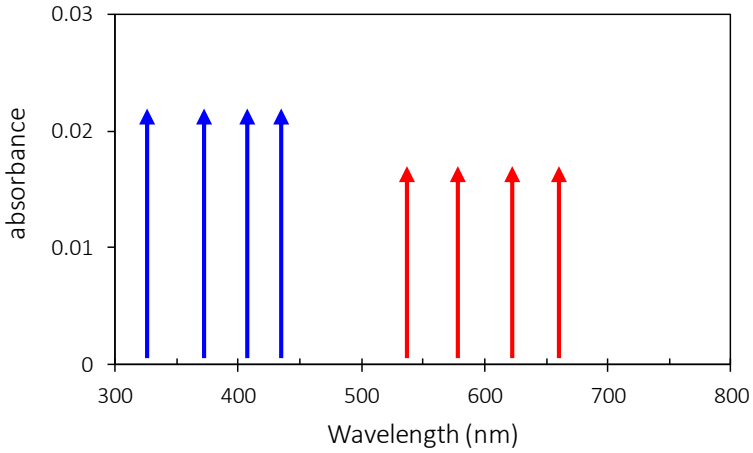

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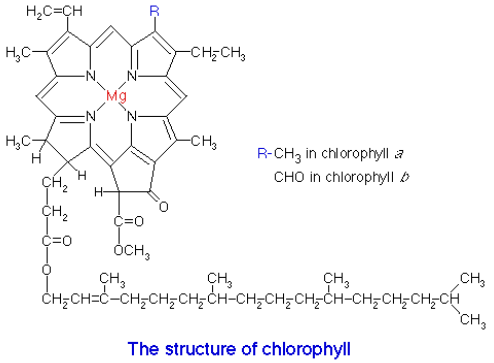

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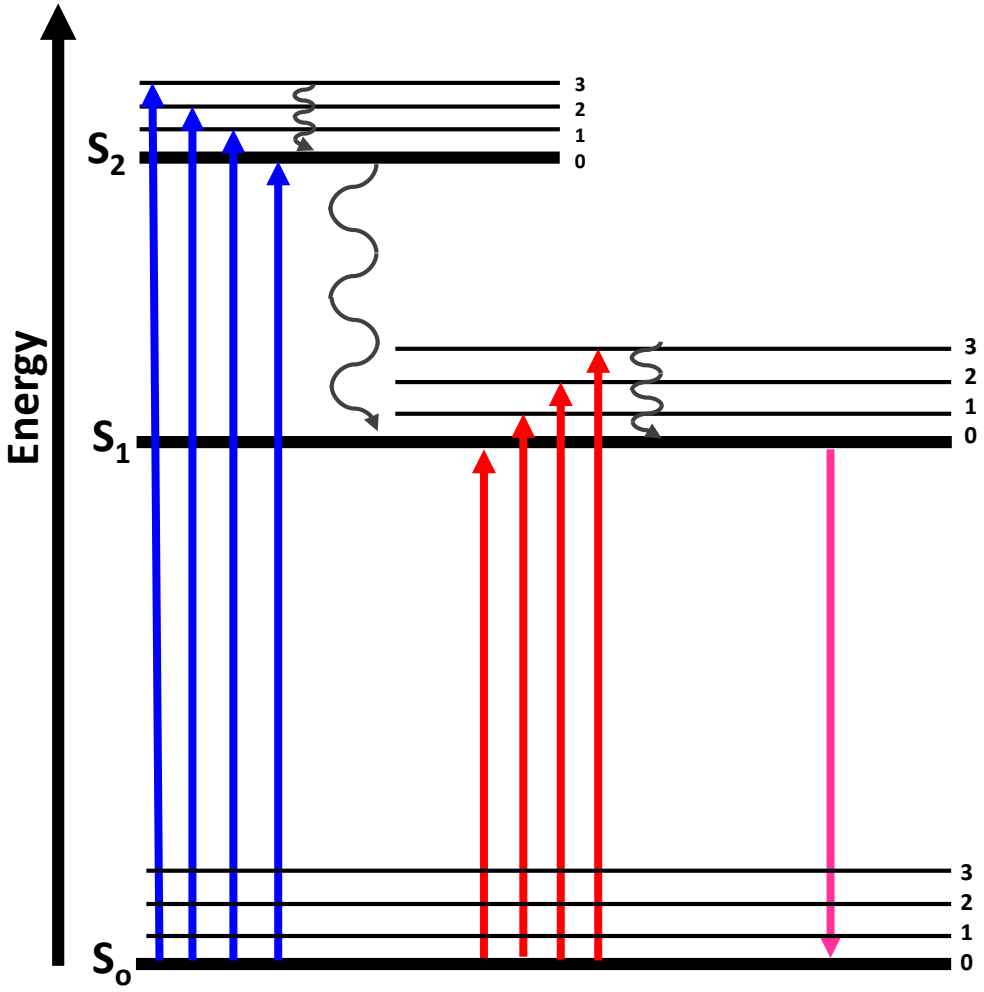
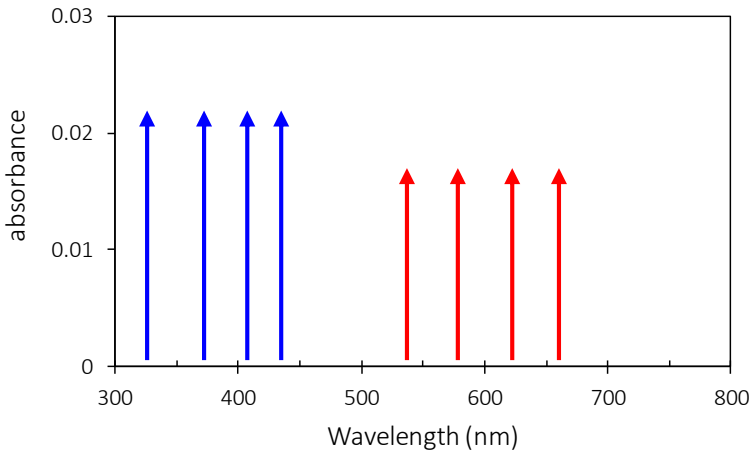


- Fluorescence from S_1 ground state to S_0 vibrational levels
- Add fluorescence to spectrum



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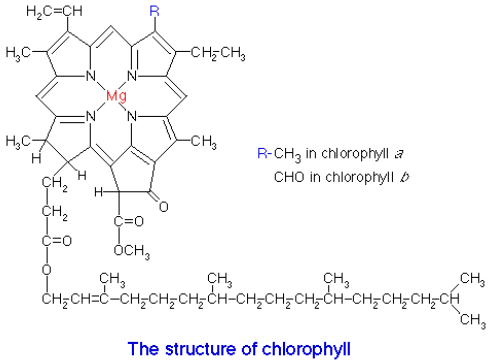

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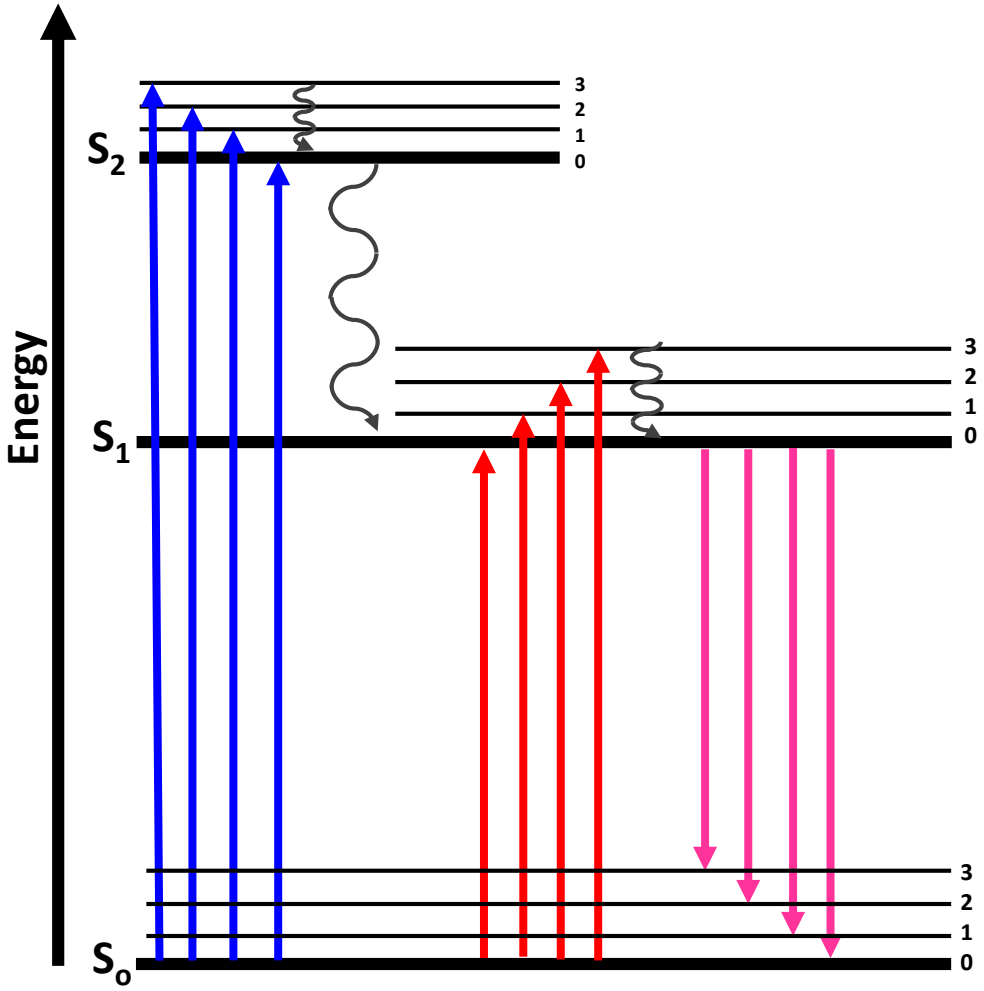

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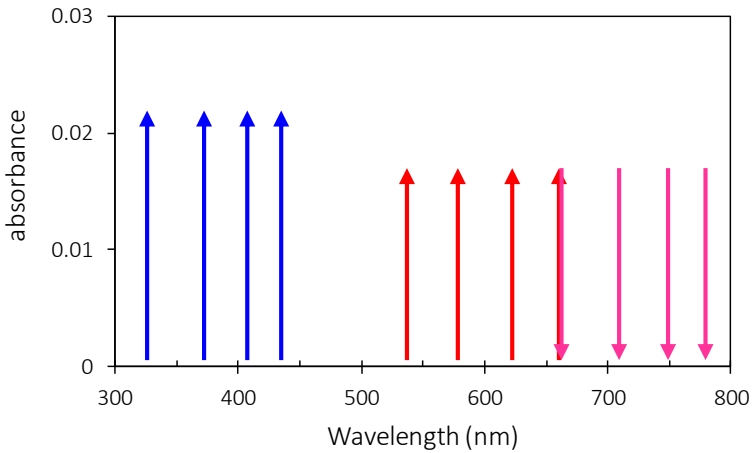

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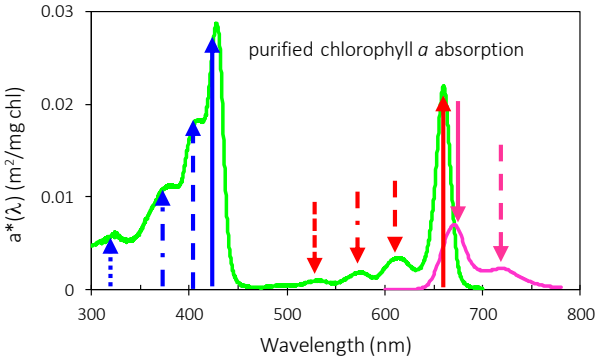
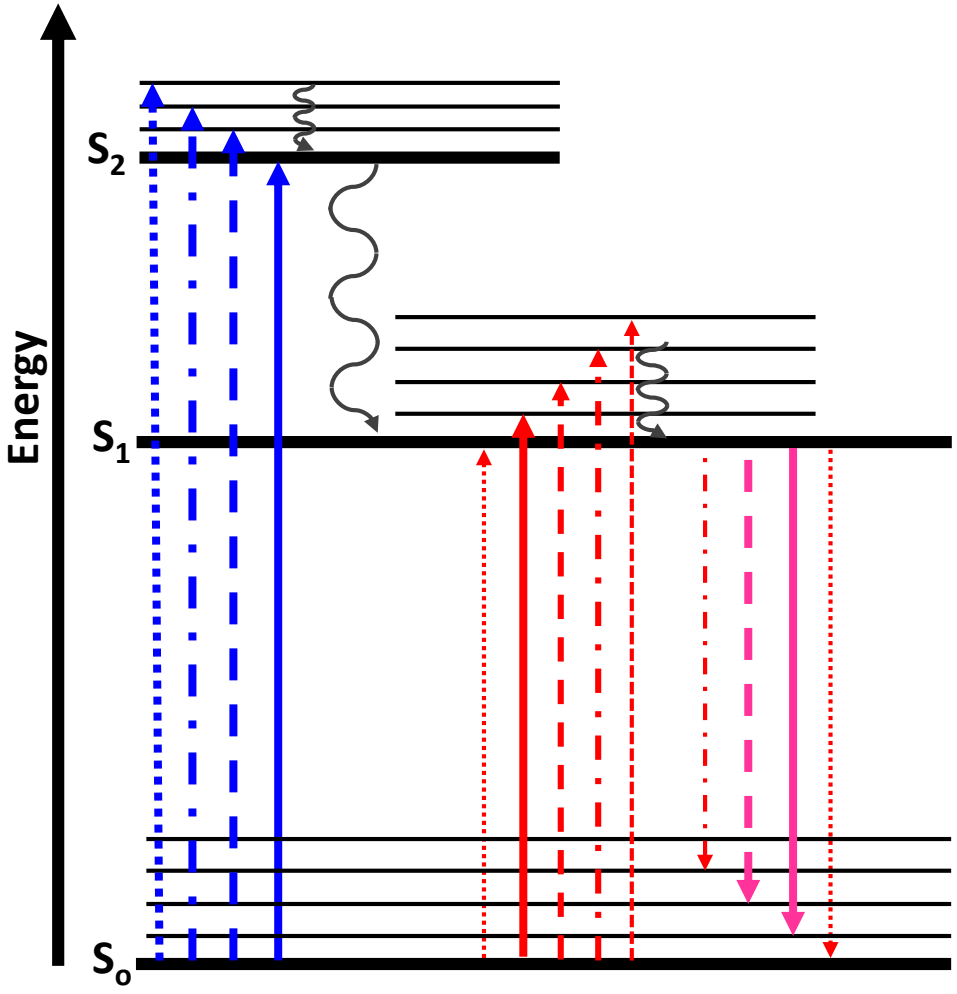
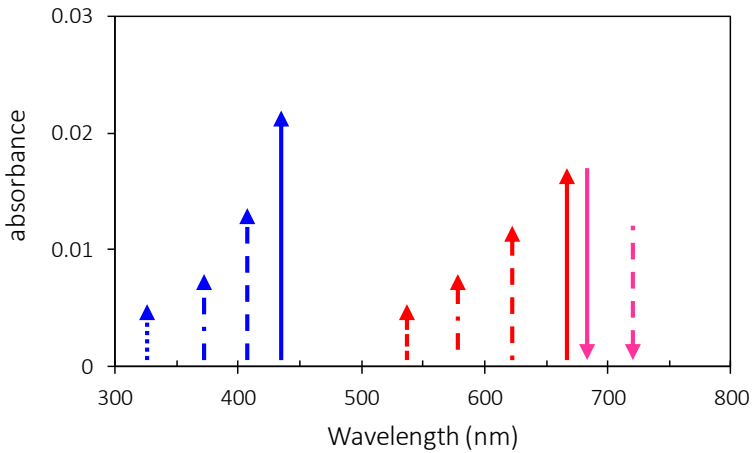
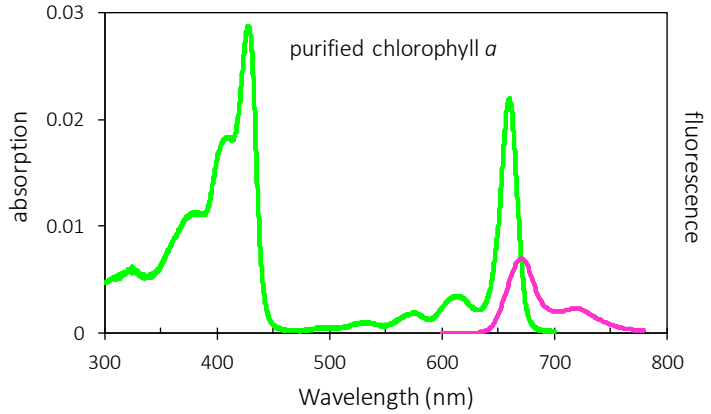




- Probabilities of occurrence in each vibrational level will determine relative absorption and fluorescence magnitudes
- Comparable for rotational levels



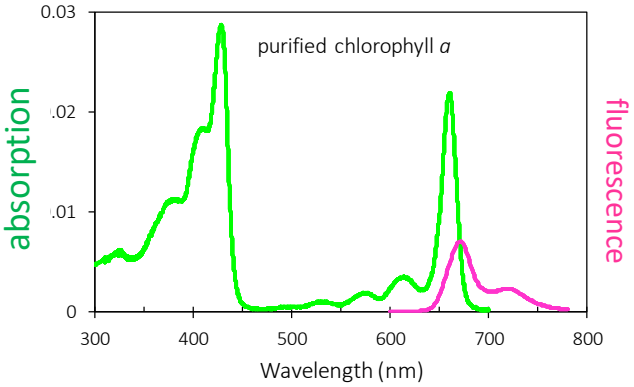
Measuring fluorescence



- Fluorescence Excitation Spectrum
  - Monitor fluorescence signal at some emission wavelength (e.g., 695 nm)
  - Excite the sample with light along the spectrum
  - Plot the magnitude of fluorescence associated with each excitation wavelength
  - Ensure or correct for constant excitation energy across spectrum
- Fluorescence Emission Spectrum
  - Excite fluorescence at some excitation wavelength (e.g., 420 nm)
  - scan the emission signal in response to excitation along the emission waveband
  - Ensure uniform detection response across emission
- Single Ex/Em (e.g., ECO sensor)
  - Excite at one wavelength (e.g., 420 nm)
  - Measure emission at one wavelength (e.g., 695 nm)
  - Calibrate to known chlorophyll concentration

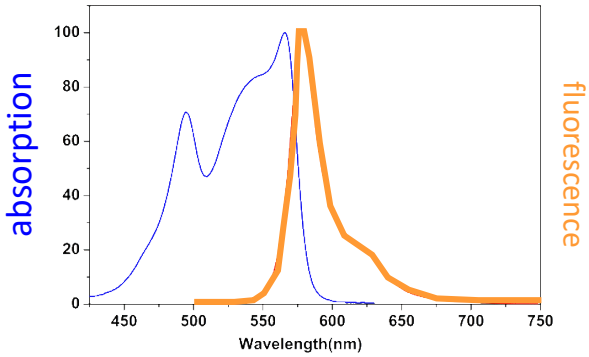
Fluorescence excitation and emission

Chlorophyll



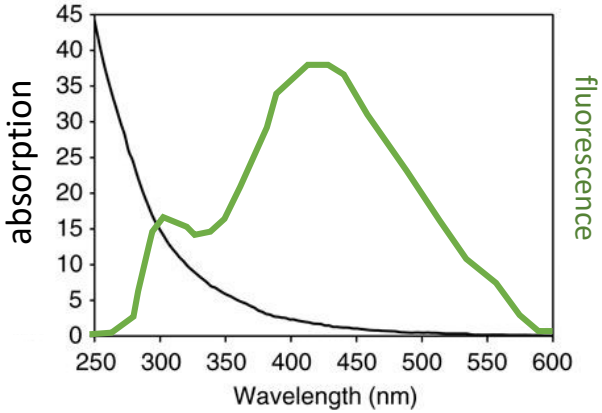
Data from Du et al., 1988.
Photochem Photobio, 68: 141-142

Phycoerythrin



http://aatbioquest.blogspot.com/2014/07/rpe-r-phycoerythrin-and-its-tandems.html

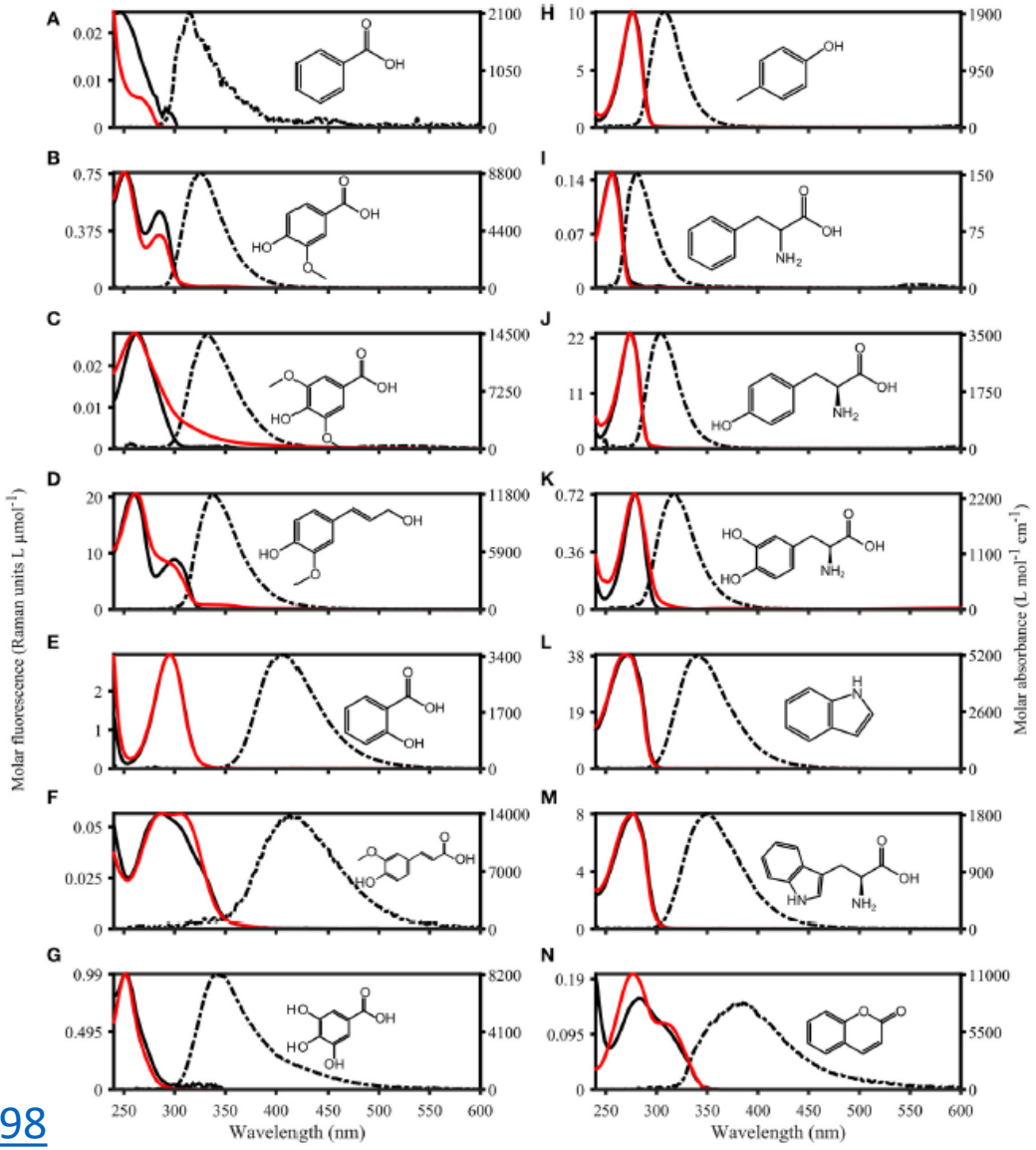
CDOM



Zhao et al. 2017. Nature 8: 15284

CDOM fluorescence

- Many fluorescing compounds
- Range of absorption, fluorescence spectra
- very complicated
- Molar absorption (red); Fluorescence (black dash)
- Longer excitation → longer emission

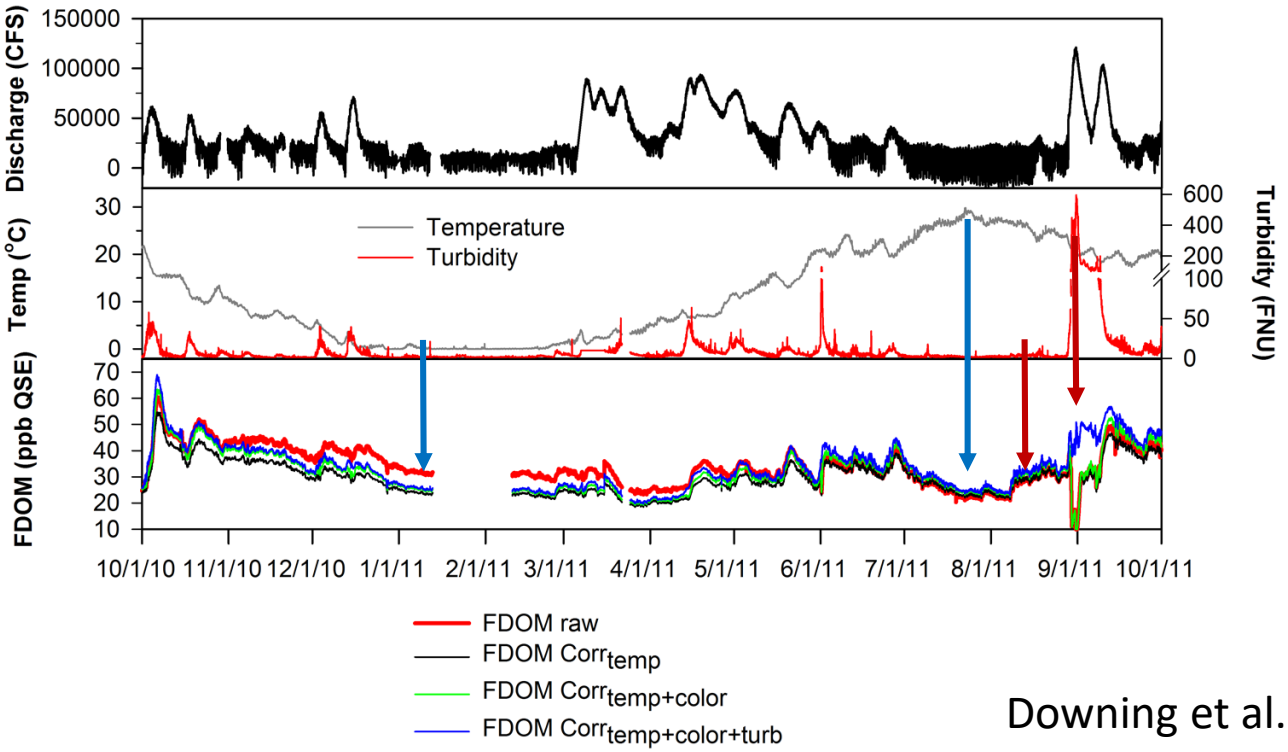


Wunch et al., 2015. Front. Mar. Sci., https://doi.org/10.3389/fmars.2015.00098

Sensitivity of FDOM to environmental parameters

- Absorption and scattering
- Temperature

$$FDOM_{corr} = FDOM_{raw} + \rho(T_{meas} - 25) / \boldsymbol{r_p}(FNU) \infty \boldsymbol{r_d}(A_{254})$$



Downing et al. 2012

Fluorescence

- What is it
- Who does it
- Physics of fluorescence
- Fluorescence Proxies
  - In vitro (in a test tube, extract)
  - In vivo (in the cell)
  - In situ (in location, ocean)
- Calibration/Validation
- Given sources of variability, what can we learn?

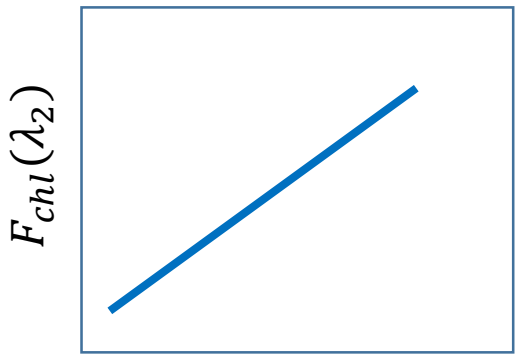
Fluorescence as proxies

- Chlorophyll fluorescence as proxy for
  - Chlorophyll concentration
  - *Phytoplankton* biomass
  - photosynthesis
- Phycoerythrin fluorescence as proxy for
  - Phycoerythrin concentration
  - *Cyanobacterial* biomass
- CDOM fluorescence as proxy for
  - “CDOM” concentration or absorption
  - Dissolved organic carbon concentration (DOC)

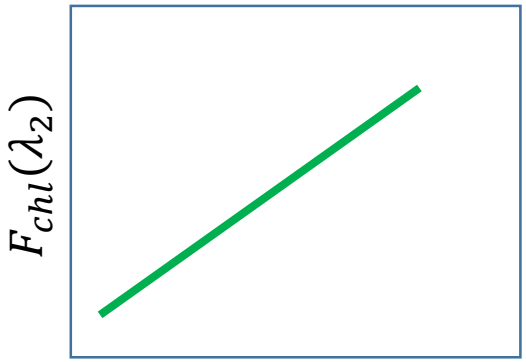
Fluorescence Signal

Efficiency factor not radiant power

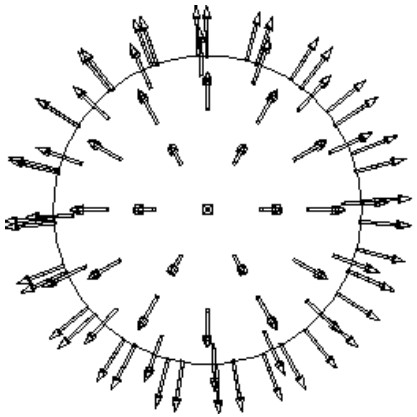
- $F_{chl}\ (\lambda_2) = E(\lambda_1) \times a_{chl}(\lambda_1) \times \Phi_f(\lambda_1, \lambda_2)$
  - Available light (spectral), $E(\lambda_1)\ (\frac{\mu mol\ quanta}{m^2 s})$
  - Chl absorption coefficient (spectral),$a_{chl}(\lambda_1)(m^{-1})$
  - Fluorescence efficiency (quantum efficiency), $\Phi_f\ \left(\frac{quanta_{fluoresced}(\lambda_2)}{quanta_{absorbed}(\lambda_1)}\right)$
  - Units on F?
  - $\left(\frac{\mu mol\ quanta}{m^2 s}\right) \times (m^{-1}) \times \left(\frac{quanta_{fluoresced}(\lambda_2)}{quanta_{absorbed}(\lambda_1)}\right)$
  - $= \left(\frac{quanta_{fluoresced}(\lambda_2)}{m^3 s}\right)$



$E(\lambda_1)$ (hold a_{chl}, Φ constant)



$a_{chl}(\lambda_1)$ (hold E, Φ constant)

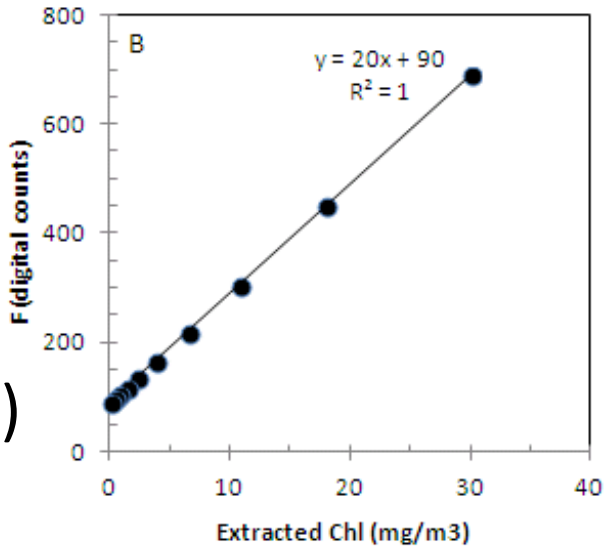


http://www.jick.net/skept/Gauss/node1.html

In vitro Fluorescence, chlorophyll proxy

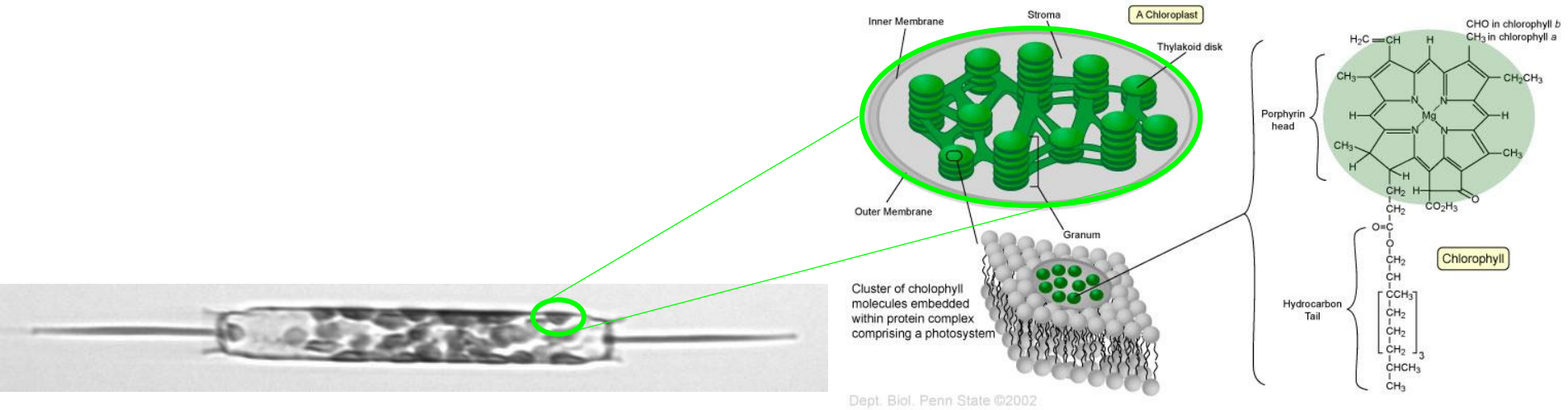
- $F_{chl}(\lambda_2) = E(\lambda_1) \times a_{chl}(\lambda_1) \times \Phi_f(\lambda_1, \lambda_2)$
- $F_{chl} = E \times a^*_{chl} \times [Chl] \times \Phi_f$

- Chl molecules in solution, $[Chl]$
- Constant mass-specific absorption, $a^*_{chl}(m^2 mg^{-1})$
- Constant quantum yield, Φ_f (no physiological pathways)
- Maintain constant E
- Fluorometers relative units, (dc or volts)
- Calibrate with Chl standard solution
- *We will do this in lab today*



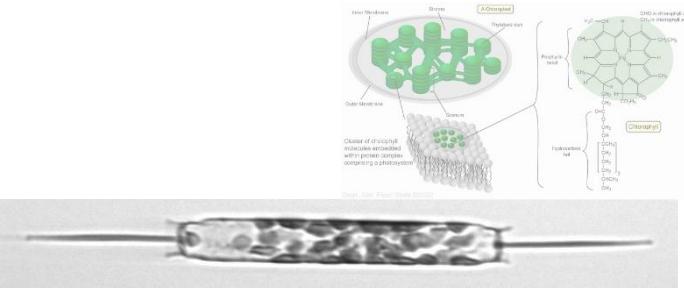
In vivo Fluorescence Physiology

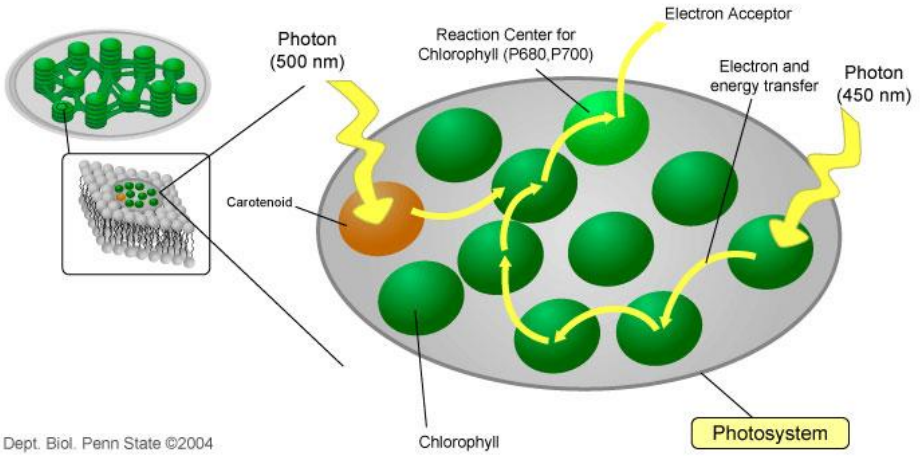
- Phytoplankton contain multiple chloroplasts, membrane-bound organelles
- The chloroplasts contain structures called grana, which are stacks of thylakoid membranes
- The thylakoid membranes have embedded pigment-protein complexes that contain light harvesting complexes of pigments and reaction centers, these are called photosystems and are the site of photosynthesis
- Light harvesting chlorophyll molecules are embedded in thylakoid membranes via the phytol tail



In vivo Fluorescence Physiology

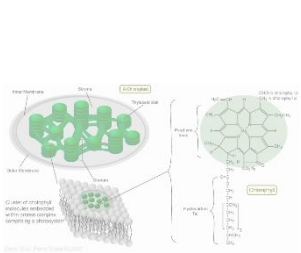
- The chlorophyll *a* molecules responsible for absorbing blue light are called the antenna chlorophylls
- Carotenoids and other chlorophylls (b and c) absorb longer wavelengths towards the green range (and red)
- Photosynthetic pigments transfer energy to the reaction center chlorophyll molecules, the transfer between adjacent molecules is an efficient radiationless and lossless dipole-dipole resonance that results from overlapping absorption spectra

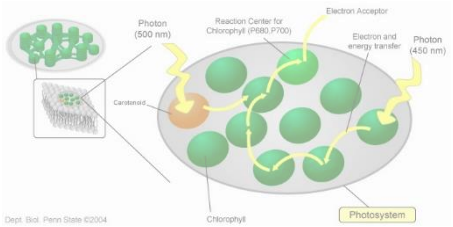


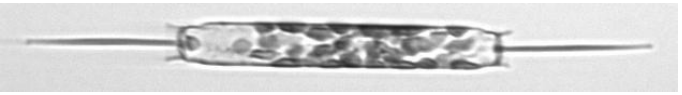


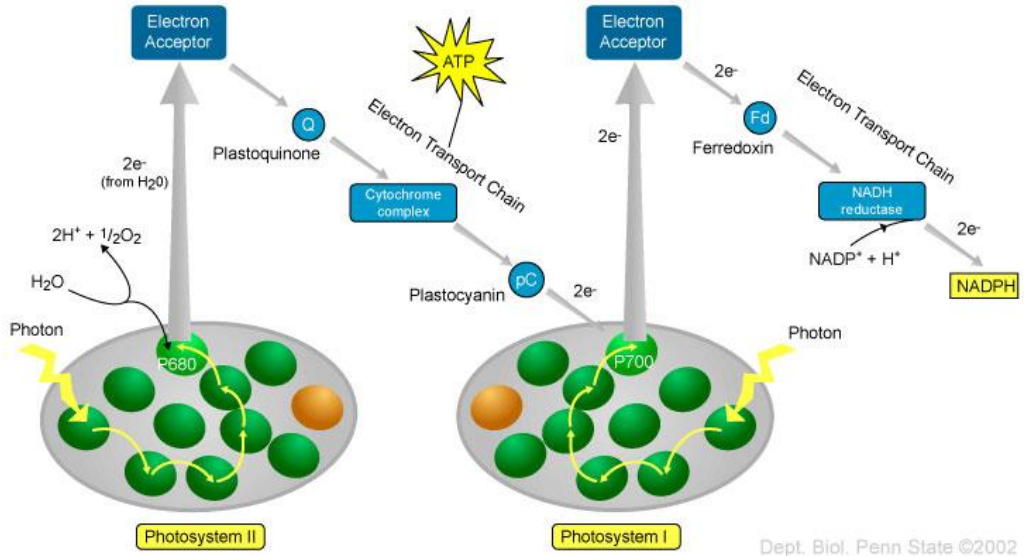
In vivo Fluorescence Physiology

- Ultimately the absorbed energy is transferred to chlorophylls in the reaction centers at a wavelength equal to either 680 nm or 700 nm for the second and first photosystems, respectively
- Once the reaction center chlorophylls are in the excited state (higher electronic levels), they pass their electrons to accepter molecules along the electron transport chains
- Water is split to replace the electron in the photosystem 2 reaction center, and oxygen is released
- The result is the formation of a reductate (NADPH) and ATP which will drive carbon fixation in the dark reactions (Calvin Cycle)



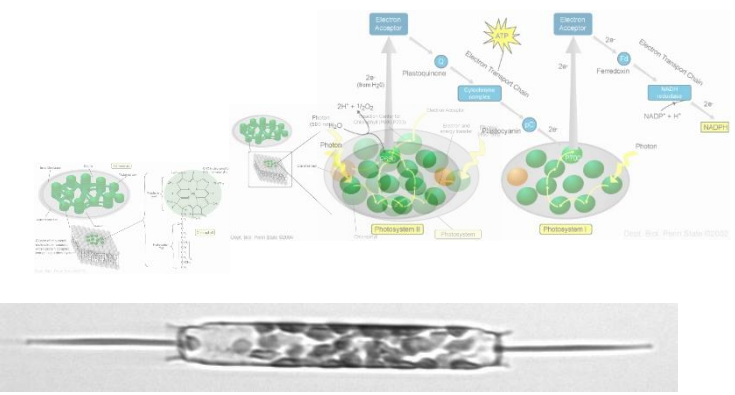


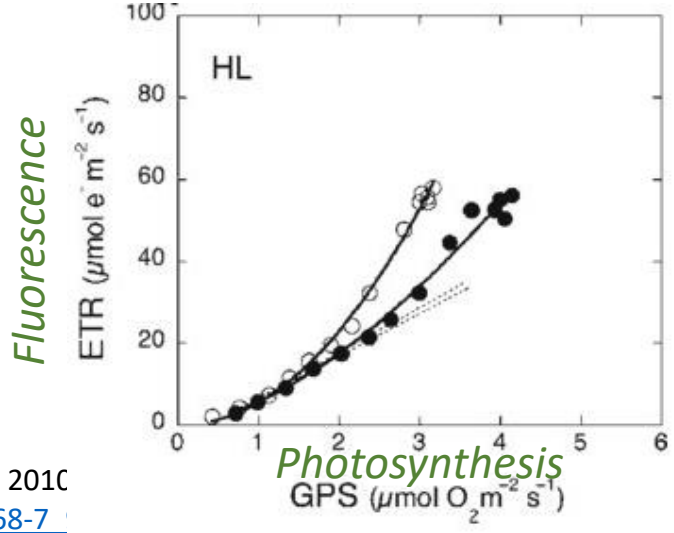




In vivo Fluorescence Physiology

- What does this have to do with fluorescence
- It is the chlorophyll molecules in the light harvesting complex that fluorescence when they cannot transfer their energy
- Initially, the more light that is absorbed, the more photosynthesis occurs *and* the more fluorescence, they vary linearly with irradiance
- As the photosynthetic rate reaches its maximal rate, excess absorbed energy is dissipated via fluorescence (and other processes), so the ratio between fluorescence and photosynthesis increases → photoinhibition of photosynthesis
- Eventually, fluorescence is also photoinhibited →non-photochemical quenching

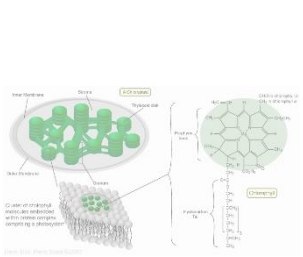


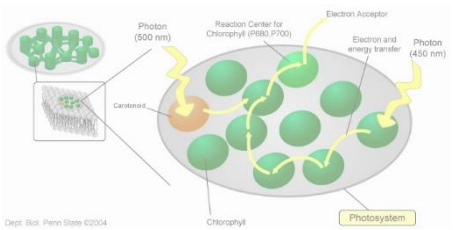


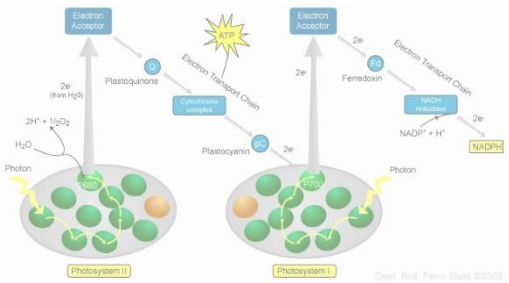
Enriquez and Borowitzka 2010
10.1007/978-90-481-9268-7_

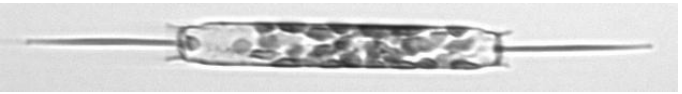
In vivo Fluorescence Proxies

- To first order, fluorescence is a proxy for chlorophyll, the more phytoplankton, the more chlorophyll, the more fluorescence
- Under limiting light levels, fluorescence is a proxy for photosynthesis (via chlorophyll and absorption)
- Under increasing light levels, photosynthesis reaches maximal rates while fluorescence can continue to increase
- Under saturating light levels, fluorescence is a not a proxy for chlorophyll, but may again becomes a proxy for photosynthesis as both quenched



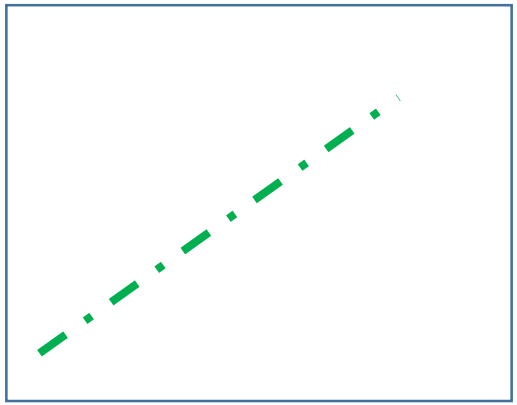






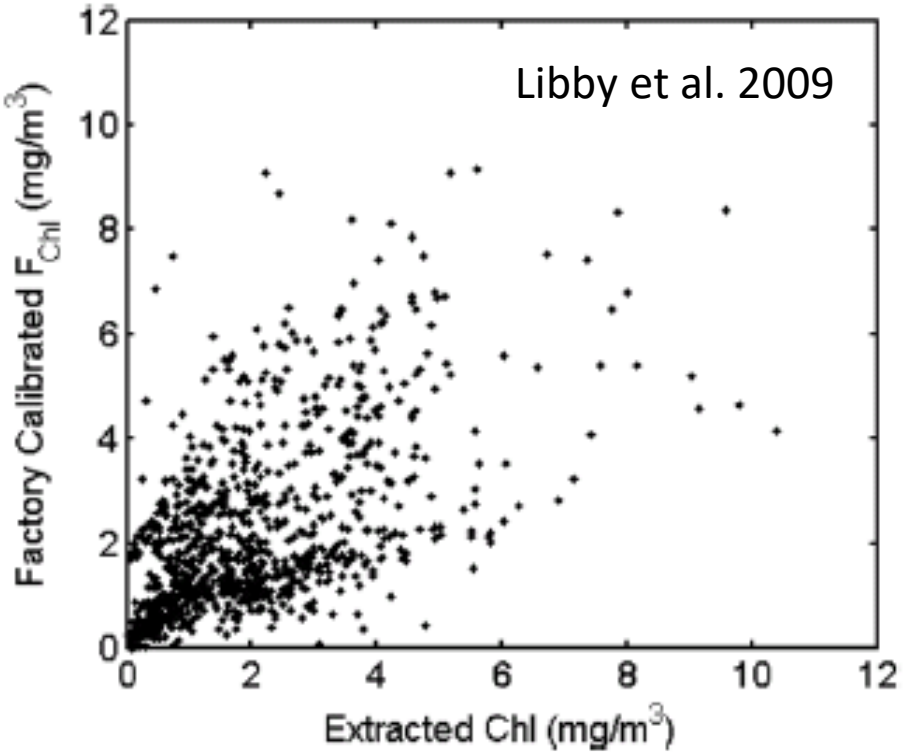
In vivo Fluorescence, chlorophyll proxy

- $F_{chl}(\lambda_2) = E(\lambda_1) \times a_{chl}(\lambda_1) \times \Phi_f(\lambda_1, \lambda_2)$
- $F_{chl} = E \times a^*_{phyt}(\lambda) \times [Chl] \times \Phi_f$
- Chl molecules bound to proteins, membranes
  - other physiological pathways
  - variations in quantum yield (~<<1-3%), Φ_f
  - Variations in mass-specific phytoplankton absorption, $a^*_{phyt}(\lambda)$
- maintain constant E
- fluorometers relative units
- Calibrate with extracted Chl samples
- How bad can it be?
- *We will do this in lab today*



How many have experienced this?

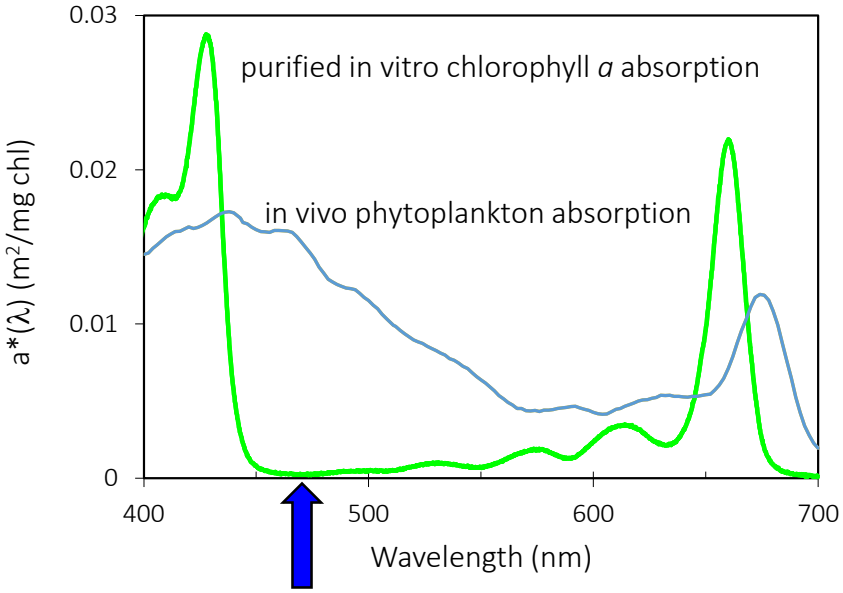
- Freshly calibrated fluorometer
- Paired *in situ* calibrated fluorescence and extracted chlorophyll concentration for validation
- ugh



- Mass Bay, 1069 paired samples

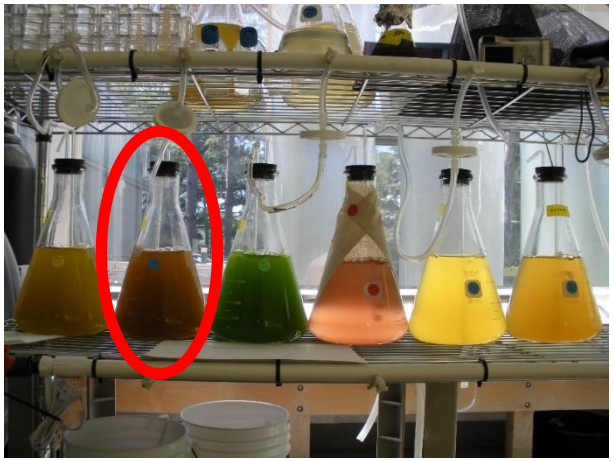
Calibration – from volts to mg/m^3

- Standards
- "solid standards" trace signal, not convert volts to [Chl]
- *In vitro* chlorophyll
  - Purified chlorophyll *a*
  - Solvent effects (wavelength shift and packaging)
  - LED excitation mismatch
    - Many sensors 470 nm excitation
    - Chlorophyll in extract doesn't absorb at 470 nm
    - 470 nm absorbed by accessory pigments, transferred to chlorophyll *a*, then fluoresced



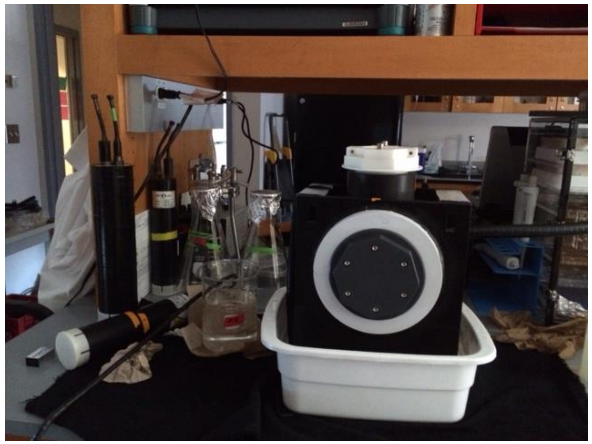
Calibration – from volts to mg/m^3

- Standards
- *In vivo* chlorophyll *a* (living culture)
  - *Traceable?*
    - Easy to culture
    - Ubiquitous
    - Robust optical properties
    - *Thallasiosira pseudonana*
    - Growth conditions
      - Replete but not inhibiting light 250 μE/m^2/s
      - 24h to discourage diel cycles/phases
      - Replete nutrients
      - Exponential growth
    - Database of chlorophyll, HPLC pigments, absorption, size, POC

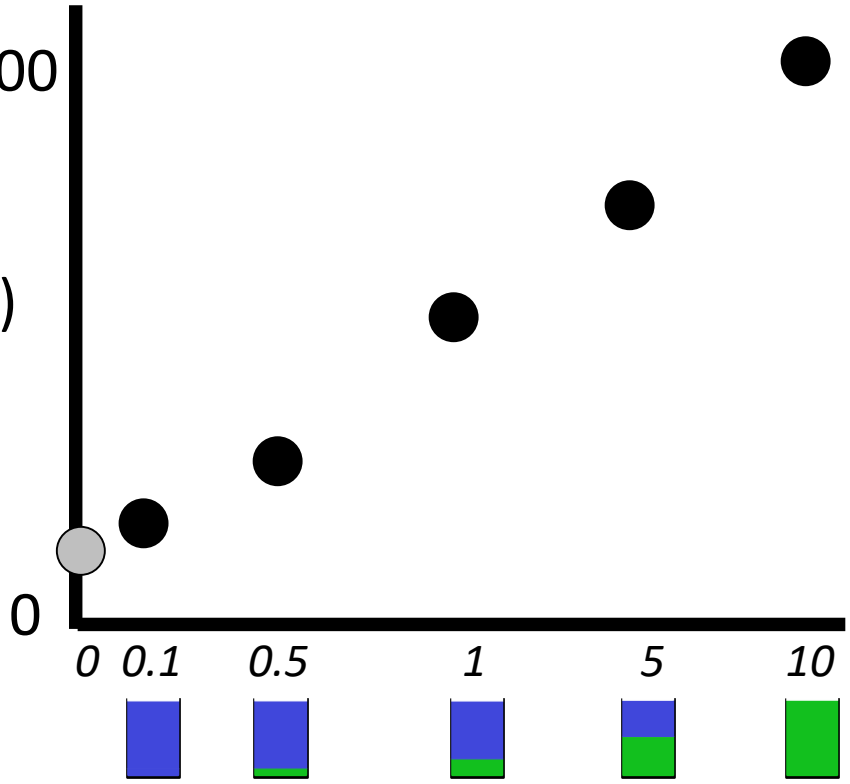


Lab Today: Calibration Standard Curve

- phytoplankton dilution series
- Measure F_{chl} of each dilution
  - Best in dark large volume casket
  - We will do beakers in dark room
- Measure extracted [*Chl*] of each dilution

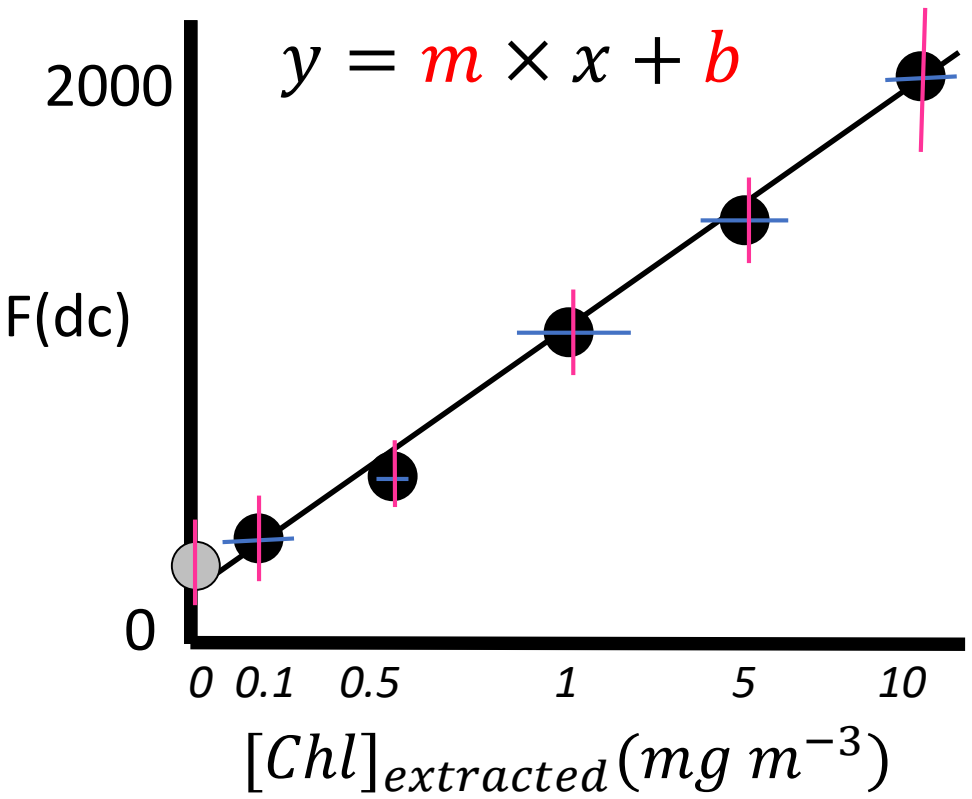


live culture



Calibration Standard Curve

- Calculate linear regression statistics (type II, std dev)
- Calibration slope,
  $m = slope = \frac{F_{meas}}{[Chl]} (\frac{dc}{mg\ m^{-3}})$
  Fluorescence yield
- Intercept, $b(dc)$, is medium blank (not the dark)
- Measure taped dark, $F_{dark}(dc)$



Equation to estimate Chl from measured fluorescence

$$Chl(mg\ m^{-3}) = \frac{F_{meas} - F_{dark}}{m}$$

Critical considerations for understanding *Sensor Uncertainty*

Characterizing your sensors is critical because you want the observations you make today to be:

- quantitative (i.e., mg chl/m^3)
- traceable (quantitatively related) to those you make tomorrow
- traceable to those you make with your other sensor
- traceable to those your colleagues make

→ climate quality data records

Fluorescence

- What is it
- Who does it
- Physics of fluorescence
- Fluorescence proxies (*physiology* of fluorescence)
  - *In vitro*
  - *In vivo*
  - *In situ*
- Calibration/Validation
- Given sources of variability, what can we learn?

There is so much more to say but that can wait until another time

- Multi-channel fluorescence as a tool for discerning phytoplankton groups
- Considerations in interpreting the fluorescence from different platforms
  - Profiling from a boat
  - Moored
  - Profiling on gliders and floats
- Non-photochemical quenching